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HLA. The monoclonal antibodies do not have necessarily to be against HLA as monoclonal antibodies against the β_2-microglobulin (β_2M) portion of HLA are also effective at alloimmune inhibition.

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Page 5, end of the paragraph beginning on page 4 at line 31 to Page 5, line 2 (Amended)

The expression "monoclonal antibodies" also meant to include without limitation murine monoclonal antibodies, recombinant MAbs, humanized MAbs, single chain MAbs, bispecific MAbs where one epitope is HLA orβ_2M, F(ab)'$_2$ and F(ab) fragments of these monoclonal antibodies.

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Page 6, paragraph beginning at line 11 to Page 7, line 14 (Amended)

DETAILED DESCRIPTION OF THE INVENTION

Previous results have shown that presensitization of donor platelets, white blood cells or whole blood with allo-specific IgG results in a diminished immune response against subsequent transfusions of platelets. To better understand the mechanism of how alloantibody presensitization results in a decreased alloimmune response, and since monoclonal antibodies do not contain contaminants as do polyclonal antibody preparations, the allospecific inhibition in the absence of the effect of the inhibitory IgG(s) can thus be examined in the present application. Murine monoclonal antibodies directed to polymorphic and non-polymorphic regions of human HLA as well as platelet-specific molecules were used in the present invention. Accordingly, it is demonstrated in

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the present application that presensitization with anti-human HLA Class I antibodies as well as β_2M-specific antibody could protect against alloantibody production to 5 subsequent untreated platelet challenges. Use of depleting (complement fixing), non-depleting, high or low FcR binding antibodies or F(ab')2 fragments of HLA-specific antibody also resulted in complete inhibition of alloantibody. This protection was not seen when the platelets were presensitized with monoclonal antibodies to CD42a (GPIX), CD32 (low affinity IgG-Fcγ receptor) or murine IgG; thus, this inhibition was therefore antigen specific and independent of complement-fixation or antibody-mediated Fc receptor dependent immunoregulatory effects. This inhibition was not dependent on HLA fine specificity, since antibodies directed at the β_2M portion of HLA class I were as effective as antibodies against any of the HLA-α regions (either polymorphic or non-polymorphic regions) of class I. In accordance with the present invention, a single regime of HLA Class I specific monoclonal antibody presensitized platelets completely inhibits alloimmunization to further transfusions and offers an approach to preventing alloimmunization.

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Page 8, (Table 1), beginning at top of the page (Amended)

Table 1
Characteristics of Sensitizing Antibodies

Antibody	Subclass	Specificity	Complement fixing	FcγRII Binding[1]	Epitope
W6/32	IgG_{2a}	HLA-A,B,C	+	+	α2/α3
MA2.1	IgG_1	HLA-A2	-	++++	α1
L368	IgG_1,k	β_2 M	-	++++	β_2M
IV.3	IgG_{2b}	CD32 (FcγRII)	+	+++	----
AN51	IgG_{2a},k	CD42a (GPIX)	+	+	----

[1] FcγRII binding of murine IgG, highest to lowest affinity: IgG1, 2b>>2a, 3

Page 10, paragraph beginning at line 9 (Amended)

Pretreatment of platelet preparations with murine IgG, CD42a-specific antibody, or FcγRII specific antibody, did not significantly decrease alloantibody production to further untreated platelet preparations compared to the positive control, untreated platelets ($p=0.92$ for mIgG, $p=0.21$ for CD42a antibody, $p=0.40$ for FcγR antibody). In contrast, platelets presensitized with either a monoclonal antibody to a polymorphic HLA epitope present on all HLA Class I molecules (HLA-A,B,C), a non-polymorphic epitope (HLA-A2), or the β_2M invariant chain (Table 1), induced no alloantibody production to further untreated platelet challenges (Fig 3; $p<0.0001$ for HLA-A,B,C and β_2M antibodies;

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A5 $p<0.002$ for HLA-A2 antibody). The total serum human IgG levels were not different in mice transfused with antibody-treated platelets compared to those receiving untreated platelets.

Page 14, paragraph beginning at line 23 to
Page 15, line 2 (Amended)

A6 Antibody-coated cells are susceptible to complement-mediated lysis or clearance by the reticuloendothelial system. While the W6/32 (HLA-A,B,C) antibody is complement-fixing, the antibodies MA2.1 (HLA-A2) and L368 (β_2M) are not, and thus complement-dependent platelet clearance is not the mechanism for the immunosuppression observed. Furthermore, the platelet-specific antibodies are complement-fixing as well, and mice challenged with these treated platelets induced a strong anti-HLA alloantibody response to further untreated platelet transfusions. Also, only the first platelet challenge was treated with the monoclonal antibodies, all subsequent transfusions being with untreated platelets.

Page 15, paragraph beginning at line 32, (Example 1), to
Page 16, line 6 (Amended)

EXAMPLE 1

Murine monoclonal antibodies

A7 The hybridomas W6/32 (IgG_{2a}, anti-HLA-A,B,C), MA2.1 (IgG_1, anti-HLA-A2/B17), L368 (IgG_1k, anti-β_2 microglobulin), and IV.3 ($IgG_{2b}k$, anti-FcγRII) were obtained from A.T.C.C. (Manassas, VA). Antibodies were used as tissue culture